Genetic Basis of Local Adaptation and Flowering Time Variation in *Arabidopsis lyrata*

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Abstract:

Understanding how genetic variation at individual loci contributes to adaptation of populations to different local environments is an important topic in modern evolutionary biology. To date, most evidence has pointed to conditionally neutral quantitative trait loci (QTL) showing fitness effects only in some environments, while there has been less evidence for single-locus fitness trade-offs. At QTL underlying local adaptation, alleles from the local population are expected to show a fitness advantage. Cytoplasmic genomes also can have a role in local adaptation, but the role of cytonuclear interactions in adaptive differentiation has remained largely unknown. We mapped genomic regions underlying adaptive differentiation in multiple fitness components and flowering time in diverged populations of a perennial plant *Arabidopsis lyrata*. Experimental hybrids for this purpose were grown in natural field conditions of the parental populations in Norway and North Carolina (NC), USA, and in the greenhouse. We found QTL where high fitness and early flowering were associated with local alleles, indicating a role of different selection pressures in phenotypic differentiation. At two QTL regions, a fitness component showing local adaptation between the parental populations also showed signs of putative fitness trade-offs. Beneficial dominance effects of conditionally neutral QTL for different fitness components resulted in hybrid vigour at the Norwegian site in the F_2 hybrids. We also found that cytoplasmic genomes contributed to local adaptation and hybrid vigour by interacting with nuclear QTL, but these interactions did not show evidence for cytonuclear coadaptation (high fitness of local alleles combined with the local cytoplasm).

Keywords: Antagonistic pleiotropy | Arabidopsis lyrata | flowering time | local adaptation | quantitative trait loci | reciprocal transplant

Article:

Introduction

Different selection pressures between environments can lead to evolution of local adaptation and often result in phenotypic differentiation between the populations. Locally adapted populations have higher fitness than nonlocal populations in reciprocal transplant experiments (Kawecki & Ebert 2004). Correspondingly, individual loci involved in local adaptation should show higher fitness for local alleles (Orr 1998). Other factors, such as different forms of selection, genomic conflict situations or genetic drift, can also lead to phenotypic differentiation. In these cases, however, the allelic effects on fitness do not consistently correspond to those of the parental phenotypes. Furthermore, plant populations are not always locally adapted (Leimu & Fischer 2008). Genetic mapping studies in the natural habitats of the populations provide valuable evidence for fitness effects of alleles from the studied populations and expression of related phenotypic traits in biologically relevant environments (Anderson *et al.* 2011a). Only a few such studies combining reciprocal transplant experiments with QTL mapping in the native environments have been reported (Verhoeven *et al.* 2004; Gardner & Latta 2006; Hall *et al.* 2010; Lowry & Willis 2010; Anderson *et al.* this issue). This type of field experiment allows evaluation of the role of different genomic regions in local adaptation.

A locally adapted phenotype can result from a combined effect of conditionally neutral loci, where a fitness effect is only seen in some environments (Verhoeven *et al.* 2004; Gardner & Latta 2006; Hall *et al.* 2010). Reduced fitness in nonlocal environments compared to the local can also be caused by antagonistic pleiotropy, in which an allele yielding higher fitness in one environment causes a fitness decrease in another environment, resulting in fitness trade-offs. Evidence for single-locus trade-offs in plants has only rarely been documented (Hall *et al.* 2010; Anderson *et al.* 2011a, this issue). In studies reported to date, local adaptation is mostly governed by conditionally neutral loci that do not have fitness effects in all environments. Recent association studies in *A. thaliana* have also found evidence for conditional neutrality (Fournier-Level *et al.* 2011). In plants, the genetic basis of local adaptation is also often tightly connected to flowering time (Verhoeven *et al.* 2008; Hall *et al.* 2010; Lowry & Willis 2010).

Flowering time has been found to be under selection in many plants, according to a meta-analysis by Munguía-Rosas *et al.* (2011). The genetic basis of flowering time should be examined both in the field and in more benign and controlled conditions, as recent studies have shown that different loci are involved in differences in flowering time in the field and in the laboratory (Conner *et al.* 2003; Wilczek *et al.*2009; Anderson *et al.* 2011b). Studies in the field are especially valuable, because the environmental cues experienced are more natural.

In addition to examining the genetic basis of local adaptation and phenological differences, experimental hybrids can be used in examining whether they show hybrid vigour or alternatively breakdown especially in later generations (Lynch & Walsh 1998; Barton 2001;Lowry *et al.* 2008). With QTL mapping, the loci contributing to hybrid fitness differences

between the parental populations can be found. When later-generation hybrids show hybrid vigour, beneficial fitness effects can accumulate either from dominance effects at QTL for many fitness components or from true overdominance. Hybrid breakdown can result not only from epistatic effects between nuclear loci, but also from such effects between nuclear and cytoplasmic genomes (Barton 2001; Levin 2003).

Cytoplasmic genomes can also have a role in local adaptation, as predicted by associations found between local cytoplasm with higher fitness (Galloway & Fenster 2001; Campbell *et al.* 2008; Kimball *et al.* 2008; Sambatti *et al.* 2008; Leinonen *et al.* 2011). Studies examining the role of interactions between QTL and cytoplasm are currently needed (Budar & Roux 2011), as cytonuclear interactions that occur as a result of co-evolution of nuclear and cytoplasmic genomes can potentially have a role in local adaptation (Rand *et al.* 2004). Cytonuclear fitness effects have also been reported in animals (Ellison & Burton 2006; Dowling *et al.* 2007; Arntzen *et al.* 2009). However, more detailed studies on the role of cytonuclear interactions in the genetic basis of local adaptation have been lacking (Budar & Roux 2011).

The genetic basis of adaptive traits in perennials has become a topic of interest recently (Anderson *et al.* 2011a). Natural selection on perennial species may influence other or additional life history traits than those found in annuals (Anderson *et al.* 2011b). Allopatric populations of a perennial outcrossing plant *Arabidopsis lyrata* (Brassicaceae) provide a valuable system for this purpose. Its distribution consists of disjunct populations of subspecies *A. lyrata* ssp. *petraea* in Eurasia and ssp. *lyrata* in North America (Jalas & Suominen 1994; O'Kane & Al-Shehbaz 1997; Al-Shehbaz & O'Kane 2002). Since the last glacial maximum, the two subspecies have recolonized the continents from distinct refugia (Koch & Matschinger 2007; Schmickl *et al.* 2010). Previous studies have revealed substantial phenotypic between-population differentiation in many traits, life history differences and local adaptation in the field (Kärkkäinen *et al.* 2004; Riihimäki*et al.* 2005; Sandring *et al.* 2007; Leinonen *et al.* 2009, 2011; Sandring & Ågren 2009; Turner *et al.* 2010; Vergeer & Kunin 2011). The availability of the genome sequence of the closely related *A. thaliana* as well as that of *A. lyrata* (Hu *et al.* 2011) enabled examining the genomic regions involved in local adaptation. Local adaptation has been documented between populations of *A. lyrata* from different continents grown in a reciprocal transplant experiment at the native sites of populations from Norway and North Carolina, USA (NC) (Leinonen *et al.* 2011). In that study, the fitness of the local population was higher than that of the nonlocal – both at the level of some of the individual fitness components and hierarchical total fitness. Different fitness components were important at the two sites, predicting that different loci might have been under selection during adaptation in the two environments. The local population started flowering earlier than the nonlocal population at both sites, indicating that adaptation to local conditions promoted flowering. F_2 hybrids included in this study had higher fitness than either parental population at

the site in Norway, while in NC the F_2 hybrid fitness was intermediate. Further, the hybrids with local cytoplasm had higher fitness, providing evidence for cytoplasmic adaptation.

Here, we apply a genome-wide approach to examine the genomic areas that have differentiated as a result of local adaptation and flowering time differentiation, those that might explain hybrid fitness differences between sites and those potentially interacting with cytoplasmic genomes. We made predictions about additive QTL effects for QTL with conditional neutrality (Fig. 1A), single-locus trade-offs (Fig. 1B) and cytonuclear coadaptation (Fig. 1C) in the two environments. Specifically, we aim at answering the following questions: first, do the QTL responsible for differences in fitness show evidence for advantage of the local alleles, especially in fitness components showing local adaptation? We predicted that as a result of differential local selection, local alleles should be associated with high fitness. Second, do these QTL show conditional neutrality (as in Fig. 1A), or do we find evidence for fitness trade-offs at the level of single QTL (Fig. 1B)? Third, are local alleles associated with early flowering in the field and what is the genetic basis of flowering time in conditions where light or nutrients are not limiting? Fourth, do QTL with dominance explain the relatively high F_2 fitness observed at the Norway field site, and are mostly additive QTL involved in NC where hybrid fitness was intermediate? Fifth, do cytonuclear interactions contribute to local adaptation and hybrid vigour and is there evidence for cytonuclear coadaptation (Fig. 1C)?

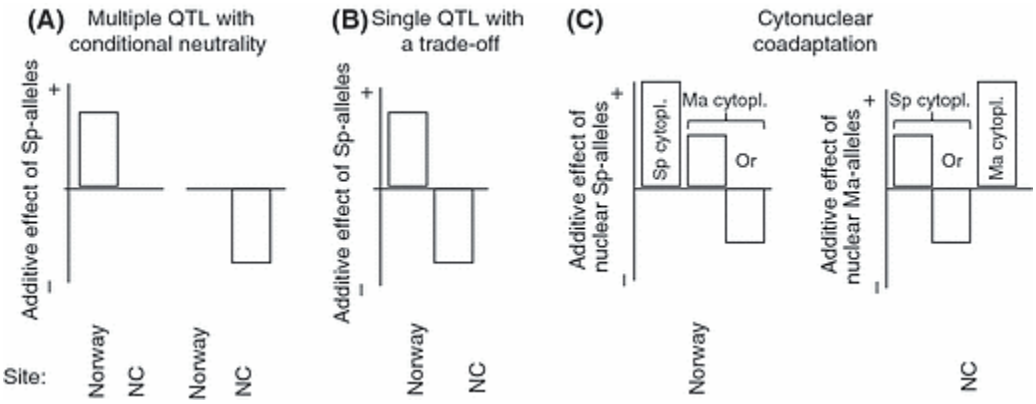

Figure 1. Predicted directions of additive effects at QTL for fitness involved in local adaptation between diverged *Arabidopsis lyrata* populations 'Sp' from Norway and 'Ma' from NC, USA, in case of (A) multiple QTL showing conditional neutrality, (B) single-QTL trade-offs and (C) cytonuclear coadaptation. Direction of effect is shown for alleles from the Norwegian Sp population at the two field sites in A and B and for alleles from the local population in C.

Methods

Plant material

Plants from two genetically diverged populations from different continents were studied. The population representing *A. lyrata* ssp.*petraea* originated from an alpine valley in Spiterstulen

Norway (61°38′N, 8°24′E; 1106 m.a.s.l.) in Europe. The North American population of *A. lyrata* ssp. *lyrata* was from Mayodan NC (36°25′N, 79°58′W; 225 m.a.s.l.) in USA. We refer to these populations as 'Sp' and 'Ma', respectively. The growing season is markedly shorter in the alpine environment, and annual mean temperature is lower in Norway than in NC. Annual mean temperature at the Norwegian site is approximately 0.87 °C (Lom, Norway, 10-year average; Norwegian Meteorological Institute) and 14.5 °C in NC (Greensboro, NC, 30-year average; U.S. National Weather Service). Although mean annual precipitation in NC is 1092 mm and only 461 mm at the Norway site, the growing season in NC is limited by intermittent summer drought. Furthermore, photoperiod during the growing season is much shorter in NC than in Norway.

To obtain F_2 progeny for QTL mapping, two plants from each of the two populations were crossed reciprocally to get two unrelated families of F_1 plants. Two unrelated F_1 plants with different cytoplasmic background were crossed reciprocally to obtain F_2 plants with different cytoplasms. This crossing design enabled us also to examine cytonuclear interactions.

Reciprocal transplant experiment in the field

To study the genetic basis of local adaptation at the natural field sites of the populations, reciprocal transplant experiments were established at the site of the Sp population in Norway in Spiterstulen and in NC at Greensboro, near the original site of the Ma population. The F_2 seeds were sown in pots with a 1:1 mix of planting soil and sand from the local area in Norway in a greenhouse. In NC, seeds were sown in Fafard germinating mix under photoperiod LD16:8 (16 h of light/8 h of dark) and kept at 4 °C for 1 week, after which they were gradually acclimated from LD16:8 to a shorter photoperiod prior to planting. The juvenile growing conditions and planting times at the two sites were chosen to increase outplanting success in each environment. Four-week-old F_2 plants from both reciprocal progeny sets were planted in the field in the end of June in Norway and in November in NC. A total of 479 F_2 plants were planted in Norway ($n_{F2Sp\text{-}cytoplasm}$ = 239; $n_{F2Ma\text{-}cytoplasm}$ = 240) and 397 in NC ($n_{F2Sp\text{-}cytoplasm}$ = 226; $n_{F2Ma\text{-}cytoplasm}$ = 171). The experiments at both sites also included plants from each parental population (Norway: n_{Sp} = 98; n_{Ma} = 120; NC:n_{Sp} = 131; n_{Ma} = 86; Leinonen *et al.* 2011).

Multiple fitness components (survival, flowering propensity, number of inflorescences, fruits and seeds) as well as flowering start date were recorded to examine local adaptation and differentiation in flowering time. This enabled us to examine the importance of different fitness components in the studied environments. Survival was recorded both in the beginning and in the end of the growing season, and flowering status (flowering or vegetative) was determined at the end of each growing season to estimate flowering propensity. The total number of inflorescences was counted, and 1–3 fruits were collected to estimate seed production. In Norway, all fruits in each plant were counted. This was not possible in NC, where the plants produced a large number of inflorescences, so the number of fruits was estimated by counting fruits from a sample of inflorescence shoots. In the fourth and fifth year at the site in Norway, only the total number of

fruits per plant was recorded as a surrogate of reproductive success. To determine the flowering start date, the date of opening of the first flower on each plant was recorded. Phenotypic data were collected from 3 to 5 years depending on the trait in Norway. In NC, we obtained data from one growing season, after which heavy mortality and new emerging seedlings prevented further collection of data. Plant material, experimental set-up and phenotypic measurements in the field have been described in detail by Leinonen *et al.* (2011). Vegetative size of the leaf rosette was also measured, but its contribution on fitness and resource allocation will be examined elsewhere (Remington *et al. in prep.*).

Experimental set-up in the greenhouse

To examine the genetic basis of flowering in benign greenhouse conditions, a total of 441 F_2 plants ($n_{F2Sp\text{-}cytoplasm} = 227$; $n_{F2Ma\text{-}cytoplasm} = 214$) were grown in a greenhouse at the Botanical Gardens at the University of Oulu, Finland. The experiment also included plants from both parental populations and F_1 individuals ($n_{Sp} = 39$; $n_{Ma} = 40$; $n_{F1} = 33$). The F_2 seeds were sown on Petri dishes in LD8:16 photoperiod. The seedlings were transplanted in pots with a 1:1 mix of peat and gravel. To induce flowering, the 6-week-old seedlings were vernalized for 8 weeks at 4 °C in LD8:16. After vernalization, the plants were grown under natural photoperiod approximately LD 22:2. Plants were watered and fertilized when needed. Flowering start date was recorded at the day of opening of the first flower. The number of inflorescence shoots was counted after 2 months from the end of vernalization. Experimental design and growing conditions in the greenhouse have been described in more detail by Leppälä & Savolainen (2011).

Genotyping

Leaf samples from the F_2 plants were collected for DNA extraction in 2005 at the end of the experiment in the greenhouse, at the field site in Norway in June 2006 and in April to June 2006 at the field site in NC. The smallest plants had to be excluded from sampling, to prevent unwanted effects of harvesting too many leaves. In 2007, some additional leaf samples were collected to increase the amount of material from the Norway site, and dried in paper bags stored in silica gel. DNA was extracted from most samples from Norway using Microlab®Star Liquid handling workstation (Hamilton). The smallest samples from Norway and all samples from the greenhouse and the NC field site were extracted using DNEasy 96 (Qiagen) or NucleoSpin (Macherey-Nagel) plant kits.

Genotyping was carried out with SNP markers that sparsely cover the genome using MALDI-TOF mass spectrometry, at the Institute of Molecular Medicine in Finland. To improve coverage, additional previously developed CAPS markers and microsatellites were also used for genotyping. The F_2 from the Norway site were genotyped for 39 SNP, 8 CAPS and 11 microsatellites, the F_2 grown in the greenhouse for 53 SNPs or CAPS markers and 23 microsatellites and the F_2 from the North Carolina field site for 40 SNP, 9 CAPS and 10

microsatellites. The linkage map was constructed using JoinMap 3.0 (Van Ooijen & Voorrips 2001). The MapChart program was used for generating the linkage map and QTL locations (Voorrips 2002). More detailed description of the markers, genotyping and linkage map construction has been documented by Leppälä & Savolainen (2011).

QTL mapping and estimation of additive effects

To identify genomic regions involved in local adaptation and phenotypic differentiation in our study populations in the different environments, we performed genome-wide interval mapping scans. These analyses were carried out separately on each phenotypic trait from each of the three environments. Both reciprocal progeny sets were analysed jointly, taking into account the effect of cytoplasmic origin by including it as an additive covariate. We used 1000 permutations to determine genome-wide LOD thresholds of $P = 0.05$ for each trait (Churchill & Doerge 1994). Because of significant block effects on plant size at the NC field site, block means for vegetative size were used as a covariate at that site. We used the *scanone* function in *R/qtl* package (http://www.rqtl.org; Broman *et al.* 2003) in *R* 2.9.2 (R Development Core Team 2009) for these analyses.

As the *R/qtl* software allows for different distributions for the phenotypic data, survival and flowering propensity were mapped as binary traits with the EM algorithm (Dempster *et al.* 1977; Broman & Sen 2009). Flowering start date and the number of inflorescences, fruits and seeds were analysed using QTL models for normally distributed traits with the Haley–Knott regression method (Haley & Knott 1992). Flowering start date at the site in NC was scaled such that day zero was the date with the earliest recorded flowering, and square-root-transformed. Survival over the first year and flowering propensity in the first summer could not be mapped at the Norway field site, because all genotyped plants had survived and flowered.

To find QTL with cytonuclear interactions, we performed additional scans using *scanone* by including cytoplasmic origin as both an additive and interactive covariate. The LOD score for cytonuclear effects was obtained by subtracting LOD scores of the additive model from the additive + interactive model. LOD thresholds for genome-wide $P = 0.05$ significance for cytonuclear effects were calculated using the differences between the peak LOD scores for 1000 pairs of permuted data sets obtained under the additive vs. additive + interactive models, using the same seed number for both sets of runs to obtain identical permutations under the two models.

To test for significance of additive effects, we used a custom script with *glm* in *R* that partitions QTL effects from outcross F_2 data into additive effects, dominance effects and differences between the two heterozygous classes (Appendix S1, Supporting infomation). This was done to test which QTL show significant additive effects (*2a*, the difference between homozygous classes) and thus contribute to between-population differences. For QTL with genome-wide significance, we performed single-locus tests at LOD peak locations for the same trait at the

other study sites or different years and for functionally related traits in the same environment using the *fitqtl* function in*R/qtl*. If the QTL main effect was significant (genome-wide $P \leq 0.05$), we tested for the significance and direction of the additive effect using the custom script. Locations of genome-wide significant and putative QTL peaks were then examined to see whether fitness differences are governed by QTL with conditional neutrality or single-QTL trade-offs. To distinguish between these possibilities, we tested whether a QTL with genome-wide significance in one environment showed (a) no significant or putative QTL in the other environment in that region (conditional neutrality); (b) a significant or putative QTL of opposite fitness effects in the other direction in that region (antagonistic pleiotropy); or (c) a significant or putative QTL with fitness effects in the same direction in that region (non-antagonistic pleiotropy). The threshold for the detection of QTL with genome-wide significance corresponded to $2a \approx 0.5–0.65$ phenotypic standard deviation depending on the informativeness of nearby markers. Single-locus tests for effects on other functionally related traits at QTL locations, however, allowed the detection of putative QTL with effects as small as $2a \approx 0.35$ phenotypic standard deviations, increasing our power to identify potential instances of pleiotropy. Corresponding tests were performed on flowering start date. To calculate the percentage of difference between the parental means explained by the additive effect ($2a$) at each QTL, inverse transformation was applied in those cases where the QTL mapping had been performed with transformed values.

We also tested the significance of dominance effects at each genome-wide significant QTL using the same custom script. Single-locus tests were performed when appropriate, as above. In case of QTL with cytonuclear interactions, we also tested whether the additive or dominance effect was only significant in one reciprocal progeny set.

Finally, we calculated Bayesian 95% credible intervals for each QTL peak (*bayesint* function in *R/qtl*). We interpreted significant overlap of QTL regions in cases where both peaks overlapped with credible intervals of the other. In cases where overlap between QTL regions was not apparent, we tested for significance of a shared QTL peak vs. two separate peaks using a likelihood ratio test, comparing the peak summed LOD score for the two traits at a common QTL location with the sum of the peak LOD scores for each trait separately. A significant chi-square likelihood ratio test statistic (d.f. = 1) was interpreted as evidence that the two traits are influenced by separate QTL (Jiang & Zeng 1995).

Results

Evidence for fitness advantage for local QTL alleles

In locally adapted populations, the plants with local QTL alleles at each field site are predicted to have high fitness (Fig. 1). Our results showed that nearly all QTL with significant additive effects supported this prediction at both field sites (Fig. 2). Effect sizes (*2a*) and the percentage that they explain of the difference between parental means are presented in Fig. 3 and all LOD

profiles in Appendix S2 (Supporting information). In many cases, the fitness advantage persisted over multiple years at the Norway site. Four QTL regions (LG1, lower part of LG2, LG4 and LG8) showed fitness advantage of alleles from the local population in more than one fitness component. In addition, there were five other QTL regions where local QTL alleles were associated with high fitness in only one fitness component in the field (upper part of LG2, LG3, LG5, LG6 and LG7).

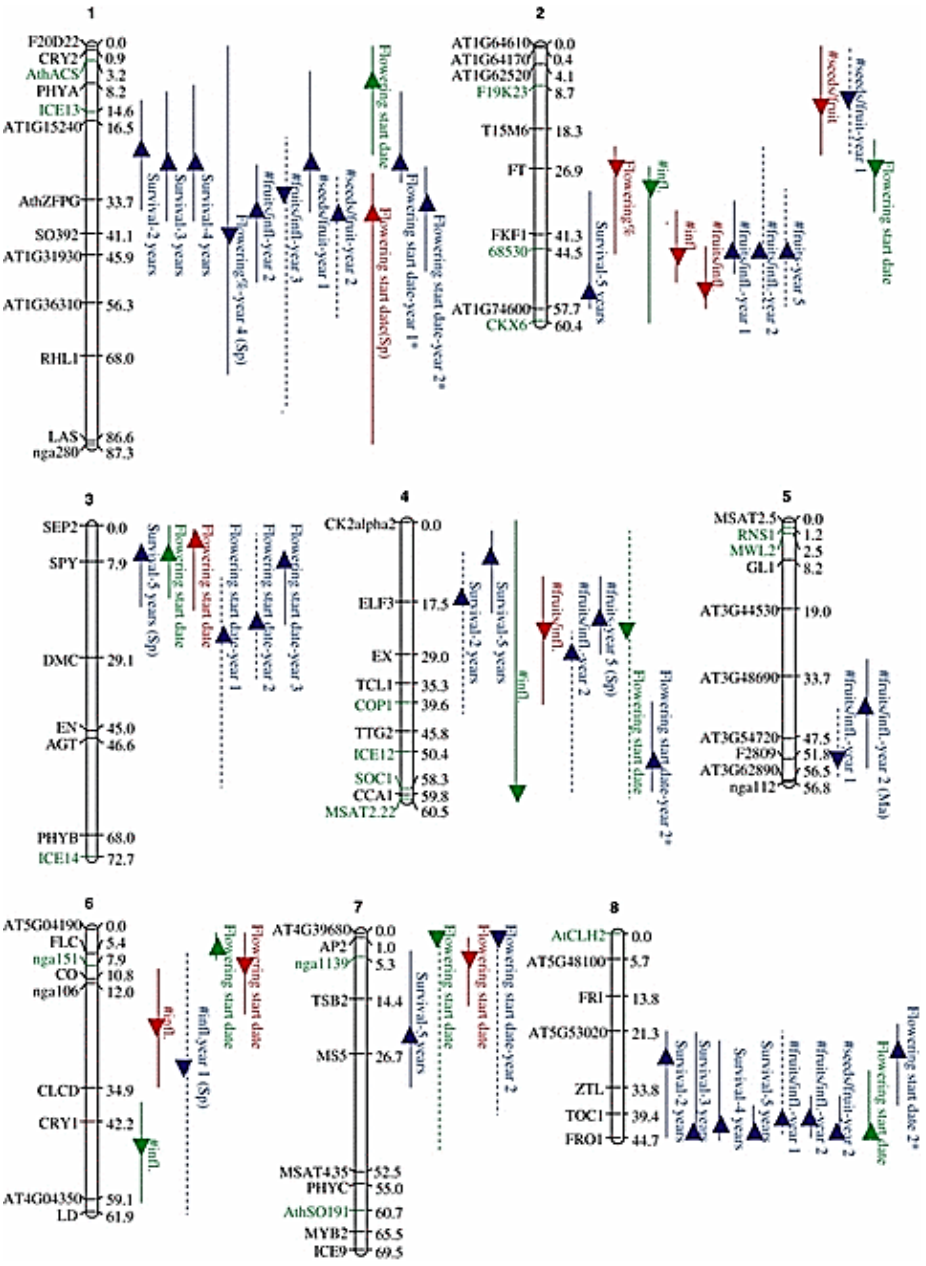

Figure 2. Additive QTL involved in local adaptation and differentiation in promotion of flowering start in Sp (Norway) and Ma (NC, USA) populations of*A. lyrata* in the three environments (green = greenhouse; red = NC field; blue = Norway field). QTL peak locations

and approximate Bayesian 95% credible intervals are shown on the right side of each linkage group. QTL with solid lines were genome-wide significant QTL ($P < 0.05$), and QTL with dashed lines showed putative QTL effects at locations of the genome-wide significant QTL in another environment, functionally related trait or same trait in a different year ($P < 0.05$). The arrow shows the direction of the additive effects of alleles from the Norwegian Sp population. In case of cytonuclear interaction, the cytoplasmic background in which the additive effect was significant is indicated in brackets. *QTL for flowering start date that were detected with the nonparametric analysis at the Norway site. Note that for traits related to flowering (including flowering start), the arrow indicates whether flowering is promoted (an upward arrow) or repressed (downward arrow) by the Sp alleles. Markers indicated with green were only genotyped for the greenhouse experiment.

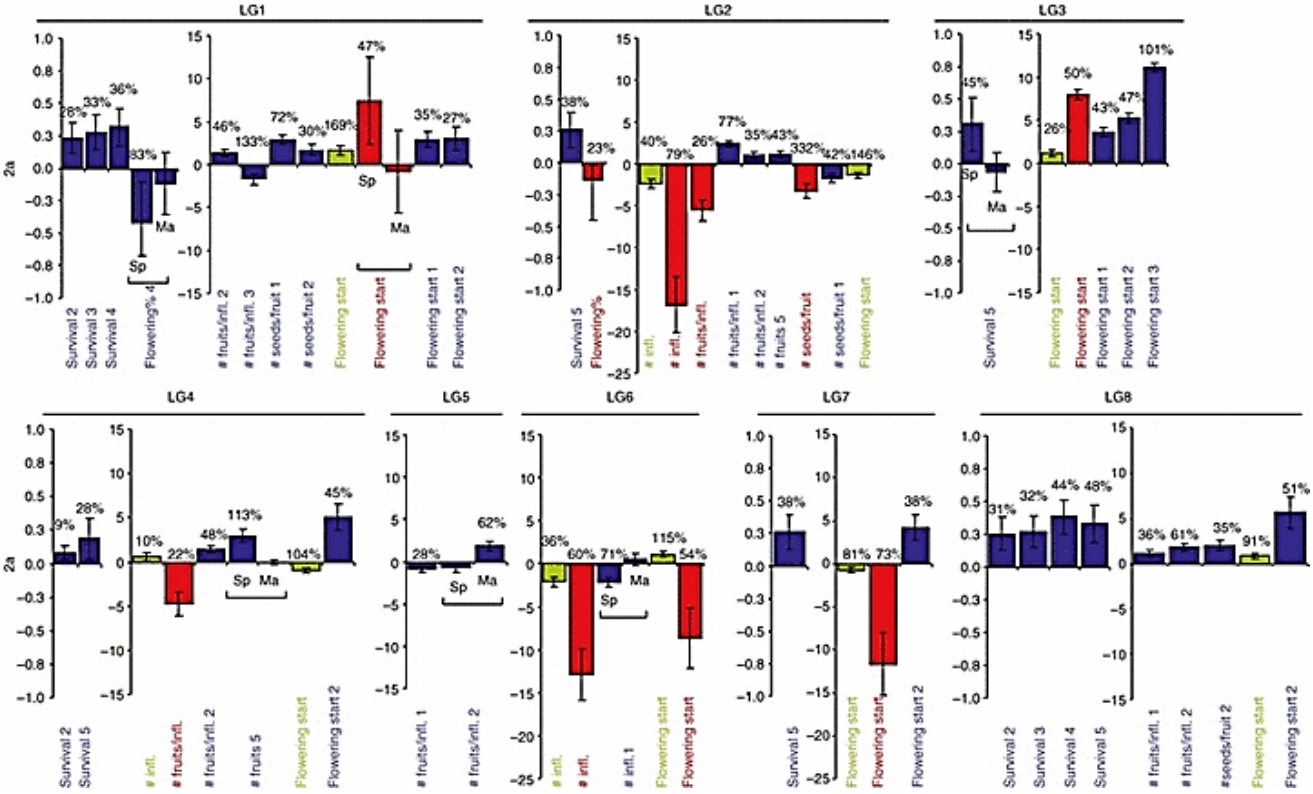

Figure 3. Size of additive effects ($2a \pm 2SE$) at significantly additive QTL (see Fig. 2) involved in local adaptation and differentiation in promotion of flowering start in Sp (Norway) and Ma (NC USA) populations of *A. lyrata* in the three environments (green = greenhouse; red = NC field; blue = Norway field). Positive: Sp alleles promote fitness and flowering; negative: Sp alleles decrease fitness and repress flowering. Percentage explained by each QTL of difference between parental population means is presented above each bar. 'Sp' and 'Ma' represent F_2 plants with different cytoplasms in QTL where additive effect was significant with either cytoplasmic background. Numbers after the trait names indicate year. Note the different scales on the *y*-axis. Note that for traits related to flowering (including flowering start), the bar indicates whether flowering is promoted (positive) or repressed (negative values) by the Sp alleles.

At some QTL, the direction of additive effect did not support the prediction of local fitness advantage at the Norway site (Fig. 2). At the QTL in LG1, there was a putative peak in the opposite direction in the number of fruits per inflorescence in the third year and in LG5 in the first year. However, at the same location in LG1, a genome-wide significant QTL in the predicted direction was found in the previous year. In the upper part of LG2 where a QTL in the predicted direction was found in NC for seed number, there was a putative QTL with corresponding effect in Norway in the first year. Alleles from the Sp population were associated with lower propensity to flower and smaller inflorescence number at all additive QTL (in LG1, LG2 and LG6) at both field sites for these fitness components (Fig. 2). In fact, these results correspond to those observed between the parental populations by Leinonen *et al.* (2011) and brought an advantage to the local population at the site in NC. The QTL for inflorescence number in the greenhouse in LG2, LG4 and LG6 showed corresponding effects (Fig. 2).

Conditional neutrality and putative fitness trade-offs

Five QTL for fitness components (LG1, LG3, LG5, LG7 and LG8) showed significant additive effects only in one environment, and thus, our results indicated that local adaptation in the studied populations is to large extent governed by QTL with conditional neutrality (Fig. 2). Two of these QTL (in LG1 and LG8) showed pleiotropic effects in the corresponding direction in many fitness components at the study site in Norway.

There were two putative cases of fitness trade-offs at single QTL regions at the lower part of LG2 and in LG4, where the number of fruits at both sites was increased by local alleles (Fig. 2). Significance of overlap was verified using likelihood ratio tests (Table S1, Supporting information). At the site in Norway, the plants with local QTL alleles in LG2 and LG4 also had an advantage in survival. In NC, the plants with local Ma alleles at the QTL in LG2 also produced more inflorescences and had higher flowering propensity. According to the additional likelihood ratio tests, the QTL for flowering propensity overlapped significantly with the QTL for fruit number in Norway (Table S1, Supporting information).

Genetic basis of flowering time differentiation

We found ten additive QTL governing differences in flowering start date (Fig. 2). At most of these QTL, additive effects were either identified only in one environment, or the direction of additive effect was in the same direction across environments (Fig. 2). In all cases at the northern field site in Norway, the local QTL alleles were associated with early flowering (Fig. 2). At four QTL (two in LG1 and one in LG3 and LG8, respectively), the Norwegian Sp alleles were associated with early flowering in more than one environment. In LG1, the QTL for flowering start in the first and second year differed significantly (Table S1, Supporting information). Interestingly, at the QTL in LG7, the local alleles were associated with early flowering at both sites (Fig. 2: note that an upward arrow corresponds to the Sp alleles promoting flowering – early

flowering start). Some QTL for flowering start in the field also overlapped with QTL for fitness components, as for example in LG1, LG3 and LG8.

In the greenhouse, there were flowering start date QTL in both directions, which was predicted as flowering start dates of the parental populations did not differ (Fig. S1, Supporting information). The QTL in the upper part of LG1 was located higher on the chromosome than the flowering start date QTL at the field sites, and the likelihood ratio tests supported distinct peaks (Table S1, Supporting information). The QTL in LG2 overlapped with a QTL for flowering propensity at the site in NC. In LG8, the location of the QTL for flowering start date in the greenhouse was significantly distinct from the QTL found at the Norway site.

QTL with dominance

We found twelve QTL with dominance at the Norwegian field site and one at the site in NC (Fig. 4). The F_2 progeny had relatively high fitness compared with the parents at the Norway site, where most of the QTL with dominance were found (Leinonen *et al.* 2011). In Norway, there was no significant additive effect at five of these QTL (in the middle and lower parts of LG1, middle part of LG4, LG6 and lower part of LG7) and the QTL in NC (LG8). These QTL suggest cases of overdominance for survival and several reproductive output traits.

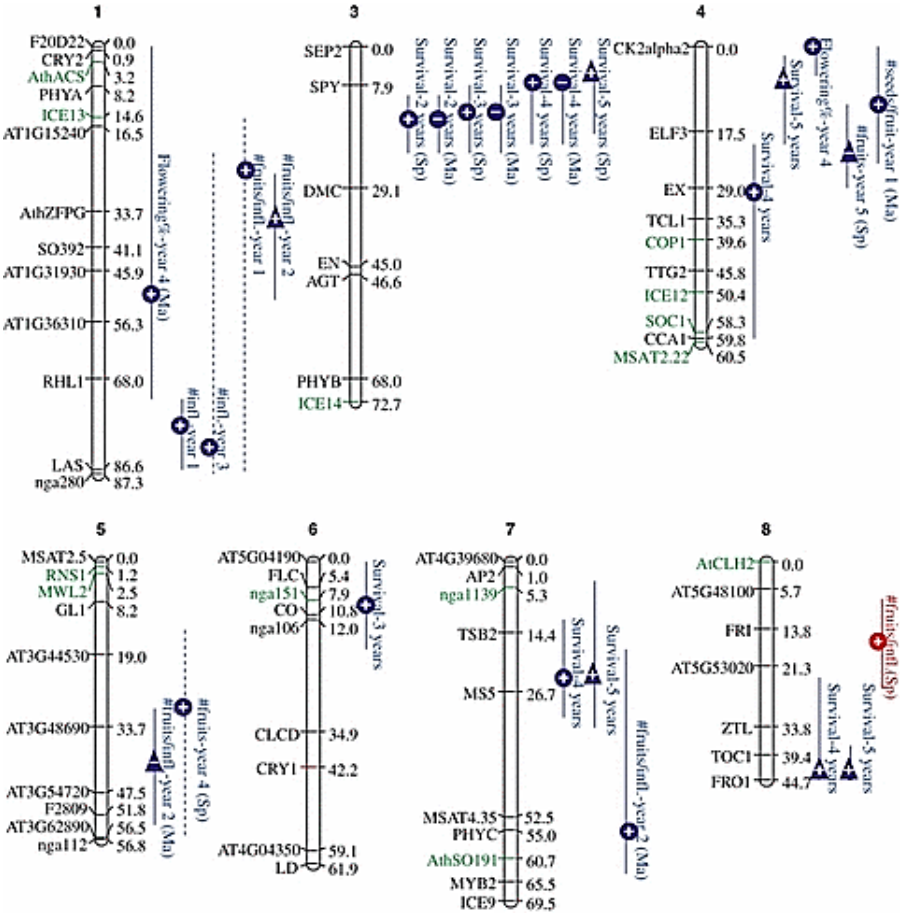

Figure 4. QTL with dominance effects involved in local adaptation and differentiation in Sp (Norway) and Ma (NC, USA) populations of *A. lyrata* in the three environments (green = greenhouse; red = NC field; blue = Norway field). QTL peak locations and approximate Bayesian 95% credible intervals are shown on the right side of each linkage group. QTL with solid lines were genome-wide significant QTL ($P < 0.05$), and QTL with dashed lines showed putative QTL effects at locations of the genome-wide significant QTL in another environment, functionally related trait or same trait in a different year ($P < 0.05$). Circles indicate QTL with heterozygotes having higher (+d) or lower (−d) fitness than the mid-homozygote-value. In case there was also a significant additive effect, the direction of the arrow shows the direction of effects of alleles from the Norwegian Sp population. If the dominance effect depended on the cytoplasm, cytoplasmic background is indicated in brackets. No QTL with dominance for the number of inflorescences were detected in the greenhouse. Markers indicated with green were only genotyped for the greenhouse experiment.

There were seven QTL with dominance (in the upper part of LG1, LG3, two at the upper part of LG4, LG5, LG7 and LG8) that also showed significant additive effects (Fig. 4). As a result, at most QTL with dominance, the heterozygotes either had higher fitness than the homozygotes or they resembled the local homozygote class with high fitness and likely contributed to the observed heterosis (Fig. 5). These QTL had relatively large effects especially on survival, which suggests that dominance in these QTL was an important factor underlying high F_2 hybrid fitness in Norway. Evidence for hybrid breakdown at the QTL level (lower fitness in heterozygotes than in the homozygotes) was found at only three of the QTL with dominance (in LG3, LG4 and LG5; Fig. 4), suggesting that negative effects of hybridization were few in our study.

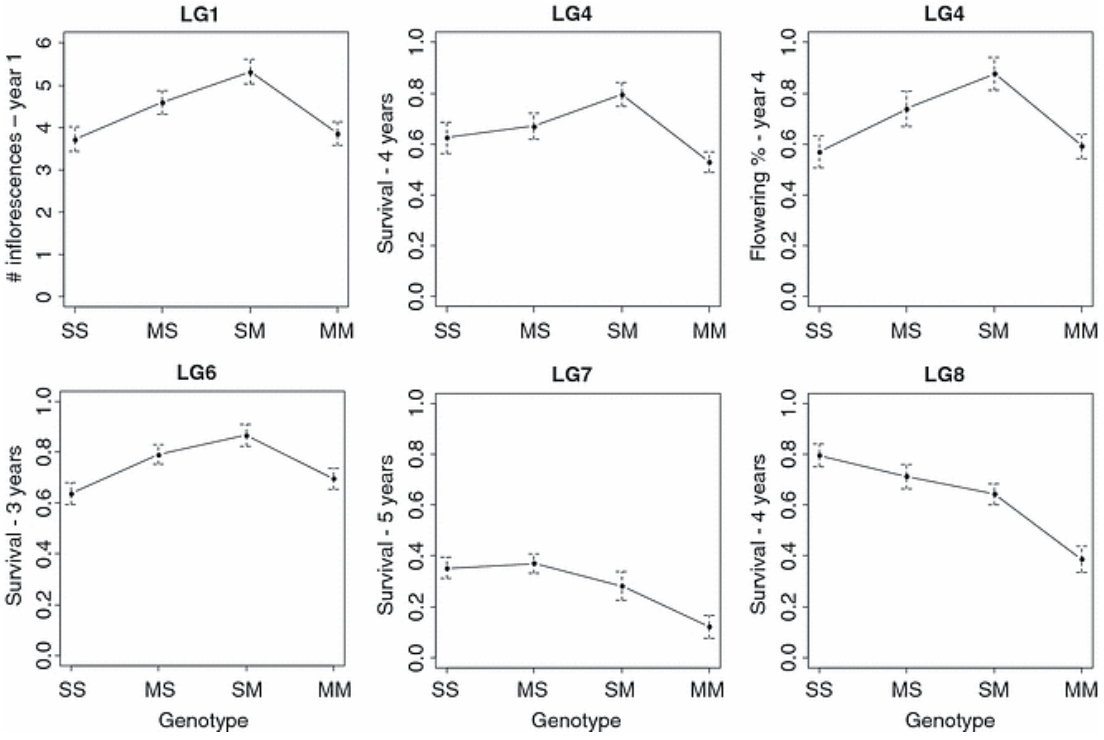

Figure 5. Genotypic means (±SE) at QTL involved in hybrid vigour in *A. lyrata*in survival, flowering propensity and number of inflorescences at the site in Norway. SS = Sp homozygotes; MM = Ma homozygotes; MS and SM = heterozygotes. 'S' represents local alleles.

Cytonuclear interactions

Our QTL results provided evidence for contribution of both nuclear and cytoplasmic genomes to local adaptation and hybrid fitness in*A. lyrata* at the field sites, but more so in Norway than at NC (Figs 2 and 4). The size of additive effects and patterns of dominance depended on the cytoplasmic background at several QTL in Norway (Fig. 6). Only two QTL (in LG3 for survival and LG4 for the number of fruits in the fifth year) supported our prediction of cytonuclear coadaptation: fitness advantage of local alleles was only seen when combined with the local cytoplasm (Fig. 6). In contrast, the QTL alleles from the Sp population in LG1, LG5 and LG6 in Norway were associated with low flowering propensity and fewer fruits and inflorescences, respectively, when combined with the local cytoplasm (Fig. 6).

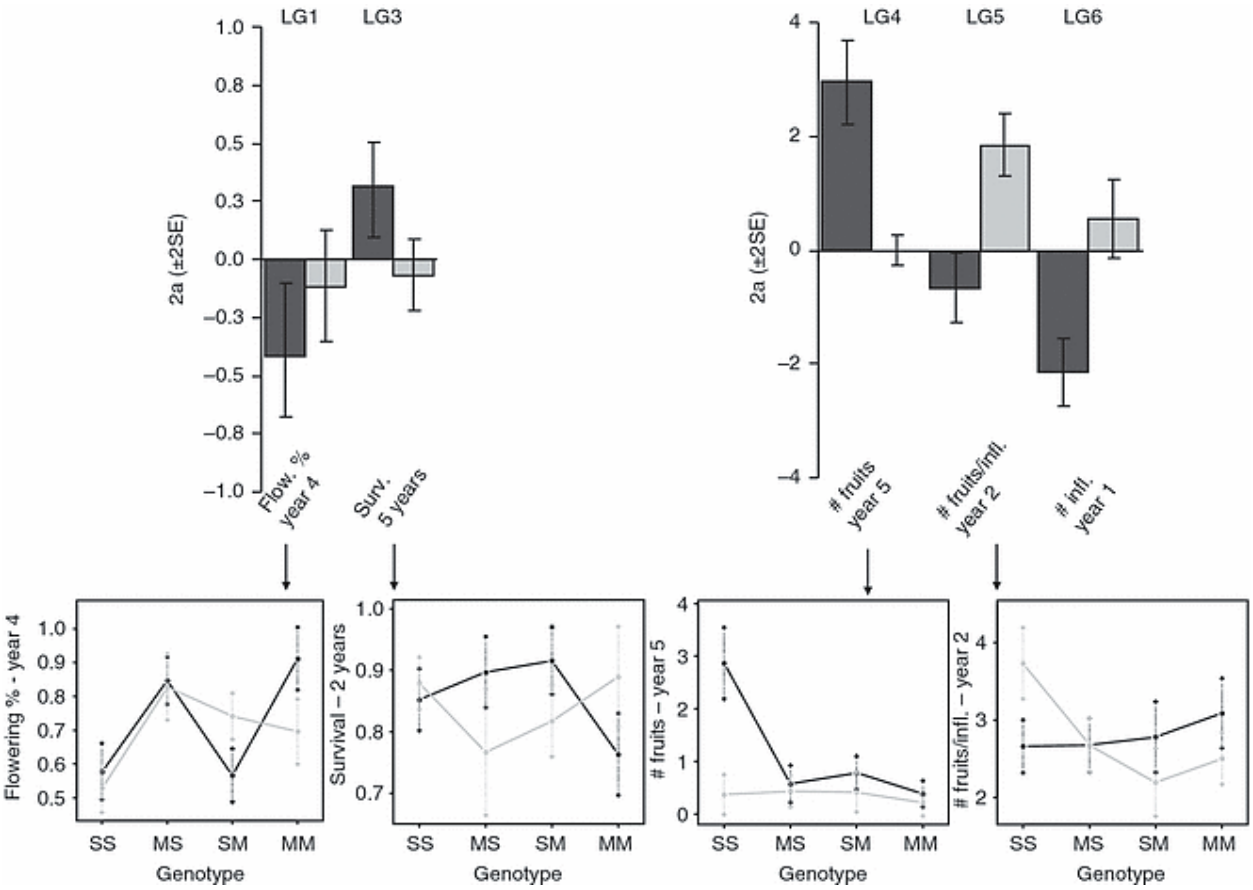

Figure 6. Additive effects of local nuclear Sp alleles (*2a* ± 2 SE) for additive QTL in *A. lyrata* in the field in Norway, where the effect size depended on the cytoplasmic background (dark grey = Sp Norway, light grey = Ma NC, USA). At four of these QTL, also dominance was affected by the cytoplasmic background (effect plots below). SS = Sp

homozygotes; MM = Ma homozygotes; MS and SM = heterozygotes. See Fig. 1C for predictions.

Cytonuclear interactions contributed also to patterns of hybrid fitness. In LG5, there was an increase in the number of fruits per inflorescence by local alleles, but only when combined with a nonlocal cytoplasm (Fig. 6), suggesting that a beneficial combination of nuclear and cytoplasmic genomes from the two populations may have contributed to hybrid vigour at the Norwegian site. At some QTL, the dominance effects depended on cytoplasmic background (Fig. 4). At the QTL for survival in LG3, heterozygotes with local cytoplasm survived better than the homozygotes, while heterozygotes with nonlocal cytoplasm had low survival (Fig. 6). At the NC site, the only QTL with dominance, affecting the number of fruits per inflorescence, was found in nonlocal cytoplasmic background in LG8 (Fig. 4).

Discussion

Genetic basis of local adaptation and flowering time

Our results support the role of conditionally neutral loci in local adaptation, as has been found also in other studies (Verhoeven *et al.* 2004;Gardner & Latta 2006; Hall *et al.* 2010). Conditionally neutral QTL can be predicted to underlie local adaptation, especially when the importance of individual fitness components depends on the environment and when no pleiotropy is involved. The locally adapted phenotype is then a result of a combination of beneficial effects of alleles at different loci in each environment. This was expected in our study case, because our study populations originate from different continents, and we have previously documented fitness advantage of the local population at native sites of both populations (Leinonen *et al.* 2011). Gene flow will not contribute to species-wide fixation of alleles that have a geographically restricted fitness advantage but are otherwise neutral. As a result, locally adapted phenotypes in separate environments can be obtained with selection on different combinations of alleles at different loci. This may result from different traits being important for fitness in different environments. Also, a particular phenotype such as early flowering can be achieved through different genetic changes in different populations. It is important to note, however, that our study included a different number of study years at the two field sites. It is possible that at the conditionally neutral QTL we observed at the Norway site, fitness effects could also have been found at the NC site after the first year, if additional data had been available.

Loci with antagonistic pleiotropy can potentially be important in determining species' ranges, as the fitness benefit they confer in one environment can be maladaptive in another environment. Single-locus trade-offs could arise as a result of contrasting selection pressures at the same genomic region, either as a result of divergent selection on the same fitness component or as a result of pleiotropic effects on different fitness components in the two environments. (Hall *et al.* 2010; Lowry & Willis 2010; Anderson *et al.* this issue). We cannot easily distinguish

whether a QTL peak in fact consists of two separate but closely located QTL, as mapping resolution is influenced by the number of recombinants (sample size), and places limitations on resolution (reviewed by Slate 2005). Furthermore, QTL with antagonistic pleiotropy could underlie fitness differences, but not be detected or verified due to limited power because they must be detected in both environments. Some of the QTL we detected had relatively wide credible intervals, and we tested for support for one vs. two distinct QTL peaks in those cases where the overlap was not apparent. Because environmental variance tends to be great especially in field conditions, it may become more difficult to detect QTL when trait values are small such as those at the field site in Norway in our study. In case of an outcrossing species, within-population variation may add additional variability possibly leading to smaller power to detect QTL. However, we believe that our sample size was reasonable for detecting at least the QTL with largest effects, such as those in the QTL in LG2 for the number of fruits per inflorescence in Norway, for example, where the difference between homozygotes (*2a*) explained as much as 77% of the difference between the parental means (Fig. 3).

Here, we applied a genome-wide approach to study genetic basis of adaptation using QTL mapping in a species with a strong signal of local adaptation based on previous studies (Leinonen *et al.* 2009, 2011). The genetic basis of adaptation has been examined recently using genome-wide association studies (GWAS), for example in *A. thaliana* (Fournier-Level *et al.* 2011; Hancock *et al.* 2011). Association studies may be confounded by a strong underlying population structure, even if methods have been developed to overcome this issue (e.g. Yu *et al.* 2006; Zhao *et al.* 2007; Kang *et al.* 2008). This is because the distribution of genetic polymorphisms affecting fitness is also highly structured by population. In species with genetically diverged populations such as those in *A. lyrata*, association mapping could still be applied at the level of individual populations (see Kuittinen *et al.* 2008). QTL mapping at a finer scale could be applied to narrow down the QTL regions found in the present study. The extensive information on the developmental biology and physiology of the related*A. thaliana* can be further used to identify the loci responsible for phenotypic variation.

The development of useful genomic tools is progressing rapidly, but understanding phenotypes and their fitness consequences still requires detailed phenotypic observations, preferably over long time spans (Ingvarsson & Street 2011). Our study is the first to our knowledge to examine the genetic basis of diverged locally adapted populations of perennial plants at their home sites across a wide geographic scale. It is important to examine QTL in multiple environments, because conditionally neutral loci underlying local adaptation will be only detected in some environments. Several studies have also documented that different genomic areas govern phenotypic differentiation in the field vs. in controlled conditions, for example in flowering time (Wilczek *et al.* 2009; Anderson *et al.* 2011b). Further, fitness should be estimated at the level of individual components, as different traits might be important depending on the environment (Nagy 1997) and some QTL underlying local adaptation might be specific to some of the components. In our study, we found that in some environments, the QTL for flowering time

overlapped with QTL for fitness. In perennials in particular, fitness should be measured over multiple years, as was carried out at our field site in Norway to account for accumulating long-term fitness effects of stress in nonlocal environments. Differentiation in allocation of resources between vegetative size and sexual reproduction can also be involved in the studied populations. This will be examined in detail at the QTL level by Remington *et al.* (*in prep.*) and was not studied here.

Environment-dependent beneficial effects of hybridization

Hybridization often yields poorly performing individuals especially in later generations (Barton 2001). We found that the F_2 hybrids between the studied populations had relatively high fitness as a result of a beneficial combination of traits (survival from the Sp parent, reproductive success from the Ma parent; Leinonen *et al.* 2011). At the genetic level, much of the hybrid vigour was also accounted by dominance at individual QTL at the Norwegian site. In principle, such findings could be due to dominance at individual loci, or result from overdominance (e.g. Charlesworth & Willis 2009). At QTL for survival, the heterozygotes resembled the local parent with high fitness. The relatively large dominance effects at the QTL for survival largely explain the surprisingly high fitness of the F_2 hybrids in Norway documented by Leinonen*et al.* (2011). Furthermore, at QTL for flowering propensity and number of inflorescences, the hybrids resembled the nonlocal Ma population that had higher trait values. However, the heterozygotes performed better than the homozygotes at some of the QTL regions (Fig. 4) and thus added to the beneficial fitness advantage gained from dominance at a QTL for survival. The proportion of cases that represent true overdominance is likely to be low though, because overdominant effects can result from linked dominant QTL of opposite direction, and otherwise, overdominance seems to rather rare (Charlesworth & Willis 2009).

Interestingly, Leppälä & Savolainen (2011) have recently documented cytonuclear male sterility in the same F_2 progeny grown in the greenhouse. In their study, there was a QTL for male fertility restorer at the upper part of LG2, whereas in our study we found a QTL for which the number of seeds was higher in plants with Ma alleles, but we did not detect a significant cytonuclear interaction (Fig. 2).

Overall, our results show that the fitness components measured here indicate beneficial effects of hybridization, but only at the Norway site. QTL with dominance were found especially for binary traits such as flowering propensity or survival. If the allelic effects act multiplicatively (as commonly assumed for these kinds of traits) and are thus represented by a logistic model, the nominal effects will appear dominant when the mean frequency is >>50% even when the underlying process is additive. At some QTL, partial dominance could be seen in the actual percentages for genotype classes, but the effect of dominance was not significant in the logit-transformed scale (data not shown).

The role of cytonuclear interactions in local adaptation

Many organellar genes have been moved to nucleus, and coordinated metabolism between the genomes has resulted in co-evolution of organelles and nuclear genomes. This suggests potential for coadaptation and epistasis between nuclear and cytoplasmic genomes (Rand *et al.* 2004). Possible cases of cytonuclear co-evolution in plants can involve proteins in which separate subunits of a protein complex are coded by both nuclear and organellar genes, such as the important enzyme Rubisco (Miziorko & Lorimer 1983). Further, gene expression in organelles is to large extent controlled by the nucleus (Nott *et al.* 2006; Woodson & Chory 2008).

A fitness advantage of the local cytoplasm has been documented in some studies, such as those in the partridge pea (Galloway & Fenster 2001), sunflowers (Sambatti *et al.* 2008), *Ipomopsis* hybrids (Campbell *et al.* 2008) and *Arabidopsis lyrata* (Leinonen *et al.* 2011). Differentiation of the cytoplasmic genome has been found to have an influence on ecologically important traits, such as water use efficiency in *Arabidopsis thaliana* (McKay *et al.* 2008). We found that the fitness advantage of the alleles from the local population was associated with the local cytoplasm at some but not all QTL in Norway, but no such effects were seen at the site in NC. Based on the results in this study, the fitness advantages of hybrids with the local cytoplasm documented in the study by Leinonen *et al.* (2011) have likely resulted from adaptation in the cytoplasmic genomes, but we did not find strong evidence for the role of cytonuclear coadaptation in our results. The cytoplasmic component in adaptation may be beneficial in the current environment, but such cytoplasmic (or maternal) effects could hinder the possibilities of successful dispersal by seeds to new areas (e.g. Galloway 2005). Future studies could also aim at identifying the cytoplasmic factors conferring local adaptation.

Conclusions

Our QTL results show that local adaptation between diverged populations of *Arabidopsis lyrata* involves mostly QTL with conditional neutrality, but possibly also single-locus trade-offs. Association of local alleles with high fitness was found especially for those fitness components that had also shown higher fitness of the local population at both study sites (Leinonen *et al.* 2011). QTL for fitness components that did not show evidence for local allele advantage showed pleiotropic beneficial fitness effects in another fitness component in some cases and can be associated with differentiation in resource allocation (Remington *et al. in prep.*). Some of the QTL for the timing of flowering overlapped with QTL for fitness. We found that beneficial and relatively large dominance effects especially at QTL for survival at the Norway site contributed to high F_2 fitness at that site. Our results also suggest that future studies should examine the role of cytoplasmic genomes in local adaptation in more detail.

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Author contributions

PHL and OS designed the experiments; PHL, JL and DLR collected the data; PHL, JL and DLR analysed the data; PHL wrote the paper with others contribution; OS acquired funding; PHL obtained a doctoral fellowship.

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Data accessibility

Data and R-scripts deposited in the Dryad repository: doi: 10.5061/dryad.93p4q.

Supporting Information

Fig. S1.Phenotypic differences in the greenhouse.

Table S1.Results of statistical tests for QTL overlap.

Appendix S1.The R-script for estimating additive and dominance effects.

Appendix S2.Genome-wide LOD profiles for QTL analyses.